Newborn Screening for Severe Combined Immunodeficiency (SCID) by Quantifying T-cell Receptor Excision Circles (TREC)

Patricia R. Slev, PhD, D(ABCC)
Medical Director, Serologic Hepatitis and Retrovirus Laboratory, ARUP
Assistant Professor, Department of Pathology,
University of Utah School of Medicine

patricia.slev@aruplab.com

July, 2013

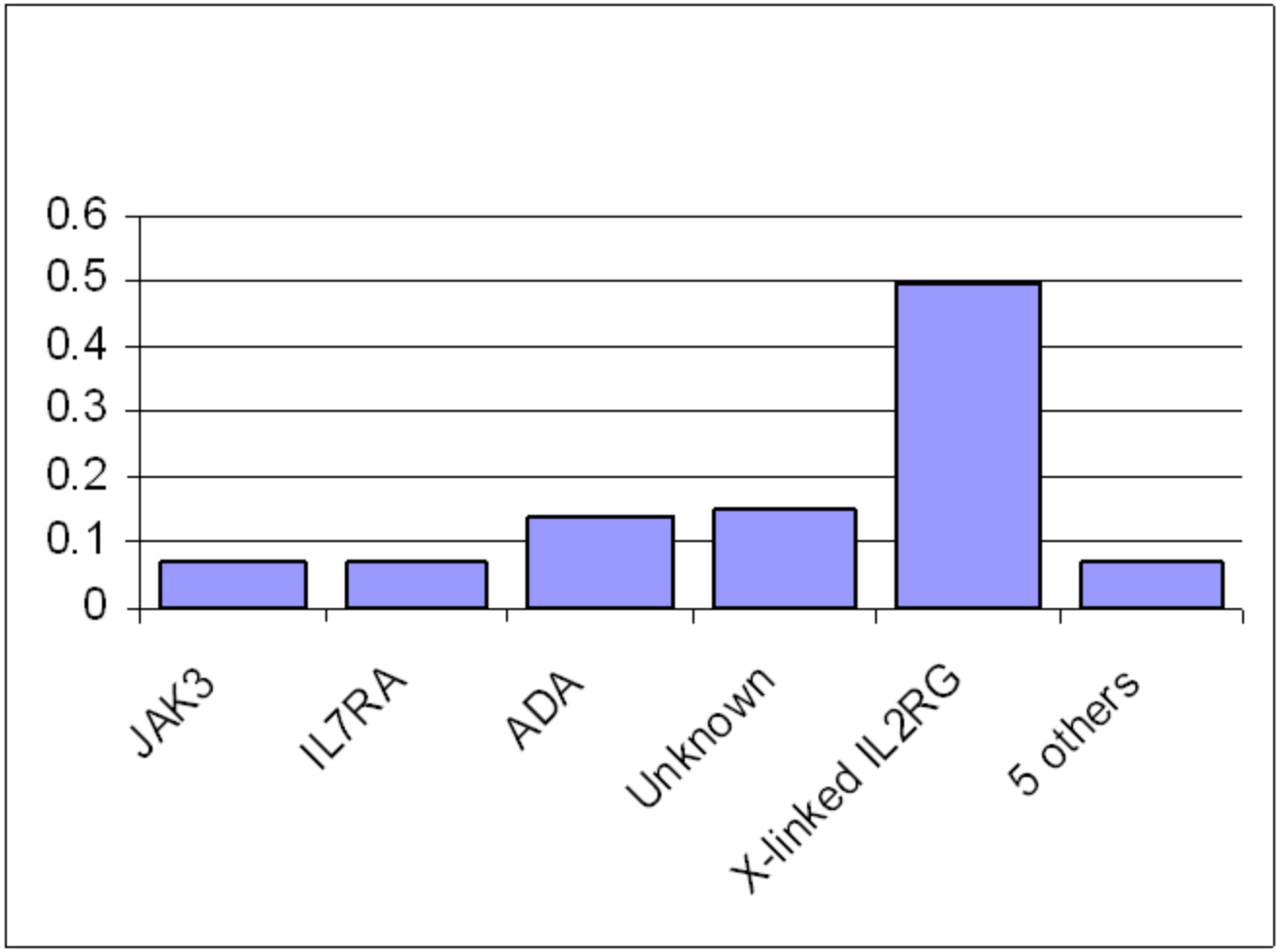
Disclosures

None

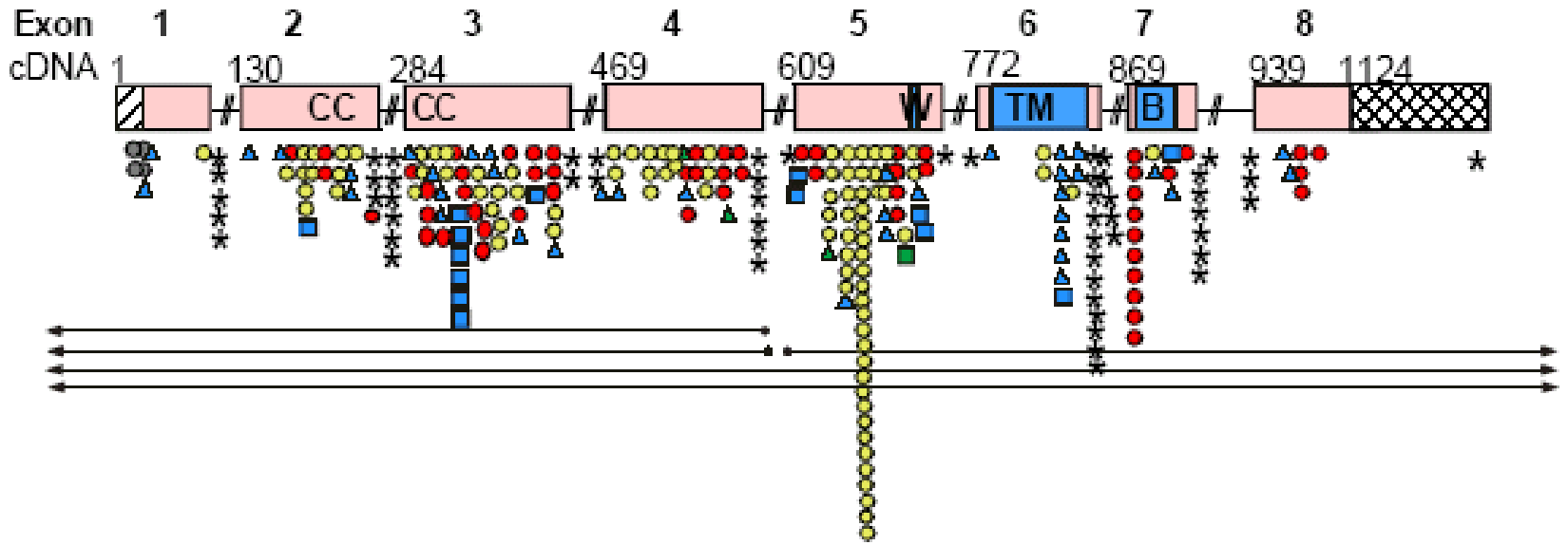
Objectives

- Understand the importance of newborn screening for SCID
- Explain why quantifying T-cell receptor excision circles (TREC) is utilized to detect newborns with SCID
- Describe quantitative real-time PCR assay for TREC
- Describe current status of newborn screening for SCID

Severe Combined Immunodeficiency Genotypes



IL2RG Mutations



IL2 RG Domains

- signal sequence
- C conserved cysteine
- W WSXWS box
- TM transmembrane
- B box1-box2 domain
- 3' untranslated

X-linked γc-SCID Mutations

- nonsense
- missense
- insertion, frame shift
- deletion, frame shift
- insertion, in frame
- deletion, in frame
- RNA processing
- large deletion
- translation mutations

Adapted from IL2RG database, Puck et al.

SCID

• IL2RG	50%	T-	B+	NK-
• JAK3	7%	T-	B+	NK-
• IL7Rα	7%	T-	B+	NK+
• CD45	rare	T-	B+	NK+
• RAG1	<5%	T-	B-	NK+
• RAG2	<5%	T-	B-	NK+
• ARTEMIS	<5%	T-	B-	NK+
• ADA	14%	T-	B-	NK-
• Reticular Dysgenesis	rare	T-	B+	NK+
• SCID, multiple bowel atresias	rare	T-	B+/-	NK+
• SCID, congenital abnormalities	rare	T-	B+/-	NK+
• Severe DiGeorge Syndrome	rare	Tlow/-	B+/-	NK+

T-cell lymphopenia

Adapted from SCID website (Duke University)

SCID Newborn Screening

- **Incidence**

  Frequency estimated (1/50,000-1/100,000) in US population

- **Treatment**

  hematopoietic stem cell transplant (HSCT), enzyme replacement, gene therapy

- **Diagnosis**

  Missed - often due to normal appearance in the newborn period
  - results in death in the first year of life

  Timing - optimizes treatment outcome and reduces morbidity
  < 3.0 months = 95% survival
  > 3.0 months = 70% survival

- **Screening Test**

  T- Cell Receptor Excision Circle (TREC)

T-cell Receptor Excision Circles (TREC)

- **Definition**

  Circular DNA formed as a byproduct of successful T-cell receptor rearrangement, which occurs in the thymus

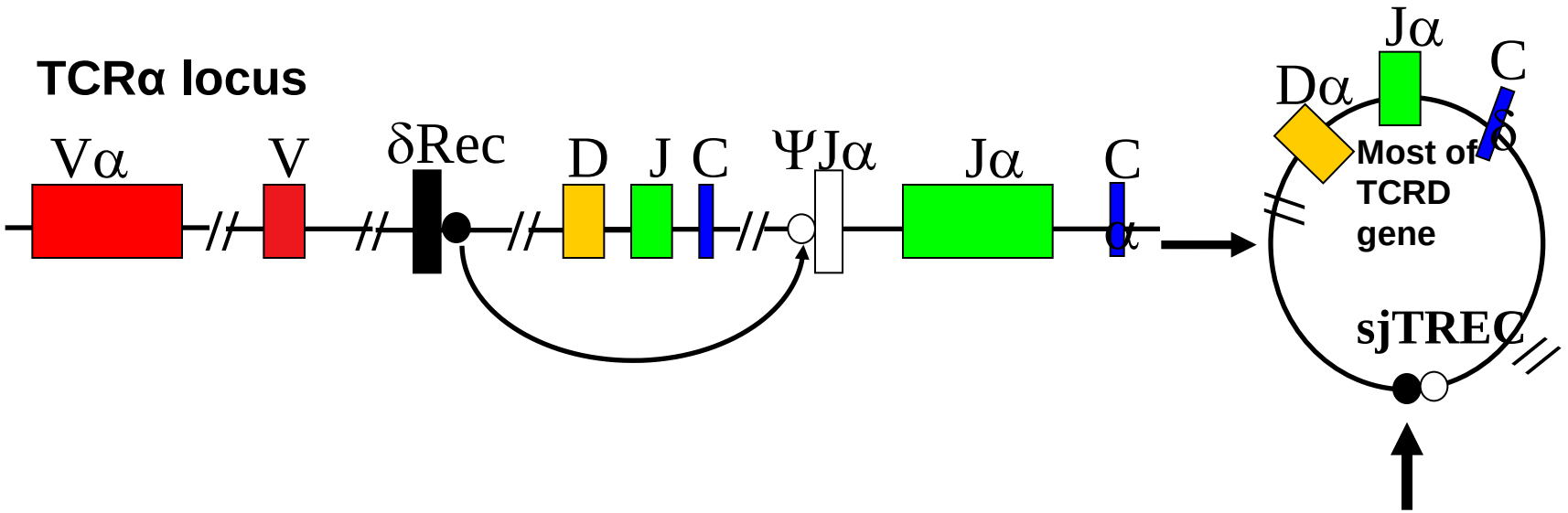
- **Characteristics**

  Present within CD4+ and CD8+ T-cells

  1 or 2 copies per cell (initially)

  Do not replicate during mitosis

δRec Signal Joint TREC



- Circular DNA formed as a byproduct of successful T-cell receptor rearrangement (TCR alpha chain rearrangement, deleting the delta locus)
- Present only in T-cells (both CD4+ and CD8+)
- δRec TREC concentrations correlate with number of newly formed, rearranged T-cells emigrating from the thymus and are a general marker for T-cell numbers

TREC as a Screen for Newborn SCID

Advantages

Maternal contamination is avoided because infants have high numbers of new T-cells and TRECs, whereas mothers have low numbers of TRECs

More easily incorporated into current newborn screening protocols than a CBC (dried blood spots)

Primary Immunodeficiency Disorders (*typically* low TREC)

Disorders	Affected Genes
Severe combined immunodeficiency (Typical SCID)	*ADA** *CD3D* (encodes CD3 delta) *CD3E* (encodes CD3 epsilon) *CD3Z* (encodes CD3 zeta) *DCLRE1C* (encodes Artemis) *IL2RG* (except p.R222C mutation) *IL7RA* *JAK3* *PTPRC* (encodes CD45) *PRKDC* (encodes DNA-PKcs) *RAG1* *RAG2* [Others]
Reticular dysgenesis	*AK2*
Coronin-1A deficiency	*CORO1A*
Thymic aplasia (complete DiGeorge syndrome)	22q11.2 deletion [Others]

Primary Immunodeficiency Disorders (*variably* low TREC)

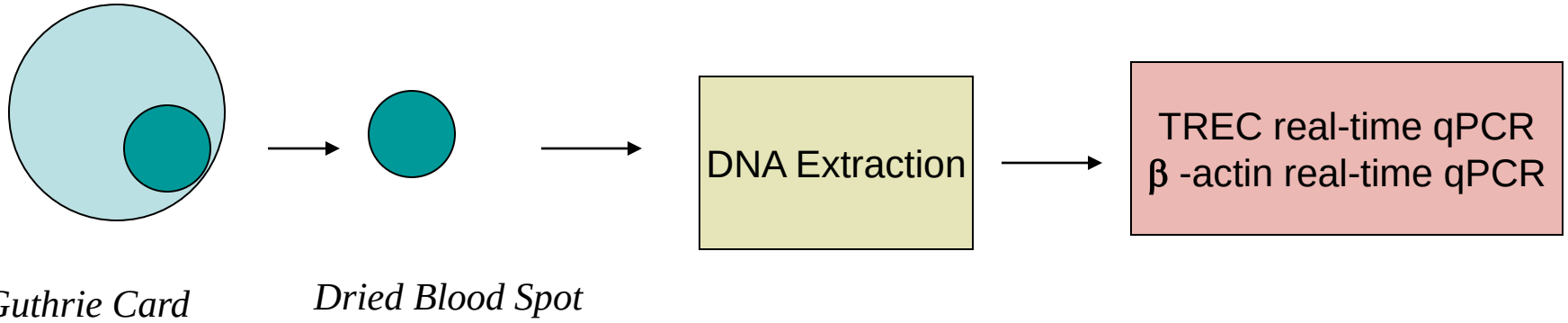
Disorders	Affected Genes
Leaky SCID/Omenn syndrome	Hypomorphic mutations in: *ADA* *DCLRE1C* *PTPRC* *IL2RG* *IL7RA* *JAK3* *LIG4* (DNA ligase IV) *RAG1* *RAG2*
Cartilage hair hypoplasia	*RMRP*
Cobalamin and folate metabolism deficiencies	*MTHFD1, MTR, SLC46A1*
Variant SCID	[Others]
Syndromes with T-cell impairment	
a. DiGeorge syndrome/22q11.2 deletion syndrome	a. 22q11.2 deletion, *TBX1*, 10p deletion
b. Cernunnos-XLF deficiency	b. *NHEJ1*
c. CHARGE* syndrome	c. *CHD7*
d. Jacobsen syndrome	d. 11q deletion
e. Small GTP binding protein RAC2 defect†	e. *RAC2*
f. Dedicator of cytokinesis 8 deficiency	f. *DOCK8*
g. Ataxia telangiectasia	g. *ATM*
h. VACTERL‡ association	h. Multiple defects
i. Barth syndrome†	i. *TAZ*
j. TAR syndrome†	j. *RBM8A* (1q21.1 deletion)
k. Down syndrome/Trisomy 21	k. Chromosome 21 trisomy
l. EEC syndrome†	l. *TP63*

Secondary Disorders (low TREC)

Prematurity
Secondary T-cell lymphopenia other than prematurity alone
Anasarca
Chylothorax
Chyloperitoneum
CLOVES* syndrome
Gastrointestinal atresia
Gastroschisis
Intestinal lymphangiectasia
Third-spacing
Cardiac surgery ± thymectomy
Congenital heart defects
Hypoplastic left heart syndrome
Neonatal leukemia
Multiple congenital anomalies/NOS
Degenerative neuromuscular disease/NOS
Presumed metabolic disorders/NOS
"Unmarkable" lymphocytes/NOS

Adapted from CLSI Document

Protocol



Guthrie Card

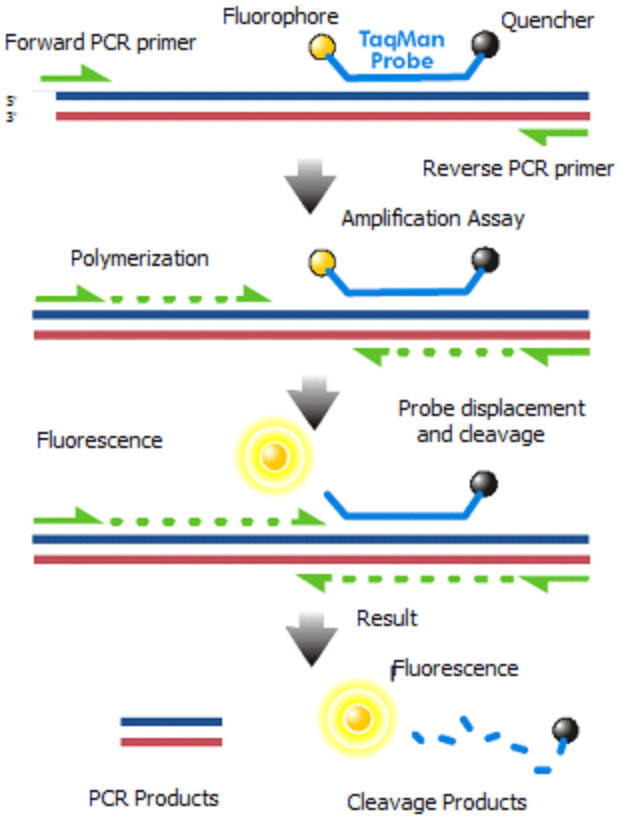
Dried Blood Spot

LightCycler 480 (Plate)

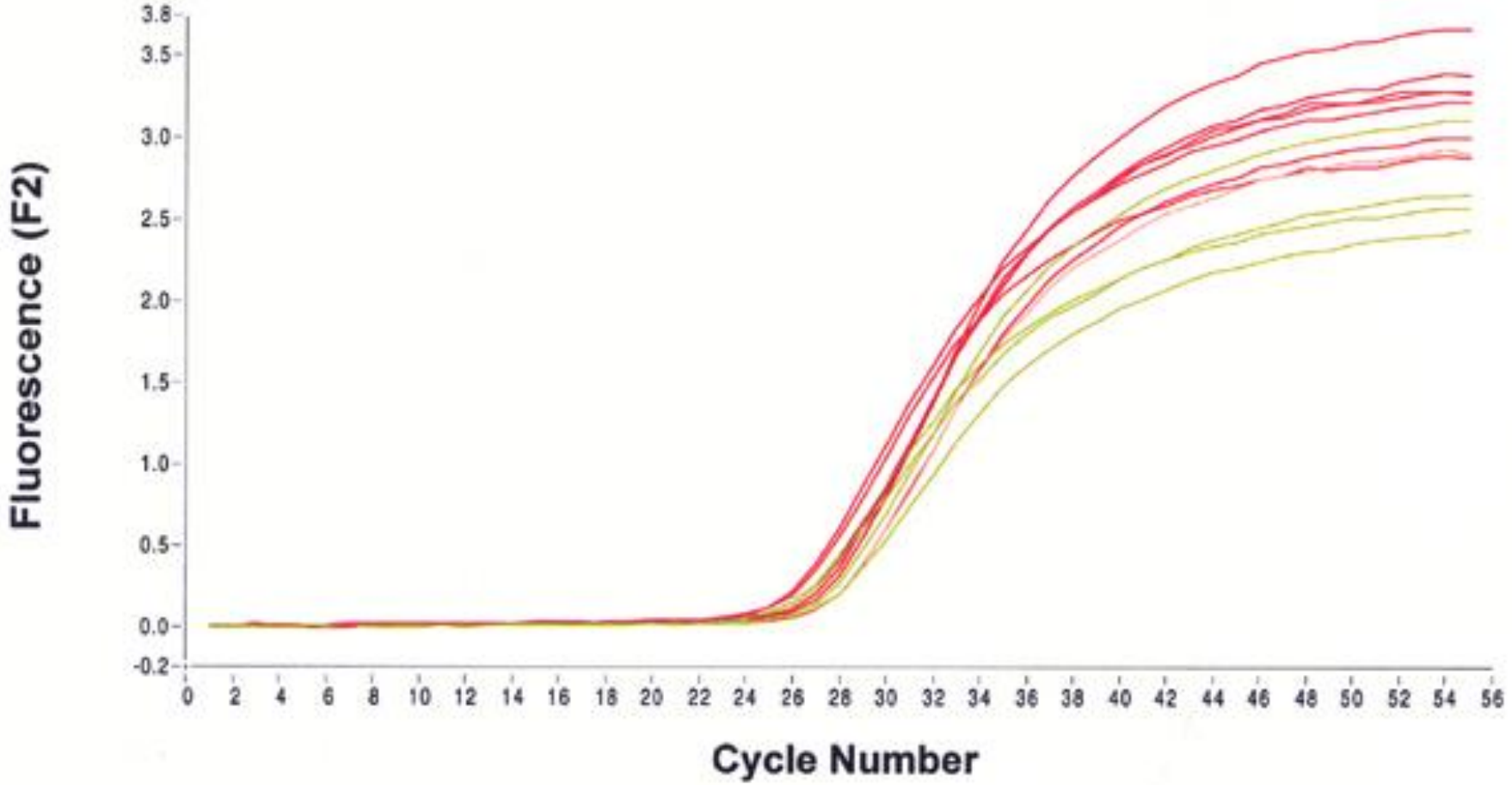
Real-time Quantitative Polymerase Chain Reaction (TaqMan)

Amplify and quantify TREC DNA
Amplify and quantify reference gene DNA
Plasmids are used as standards

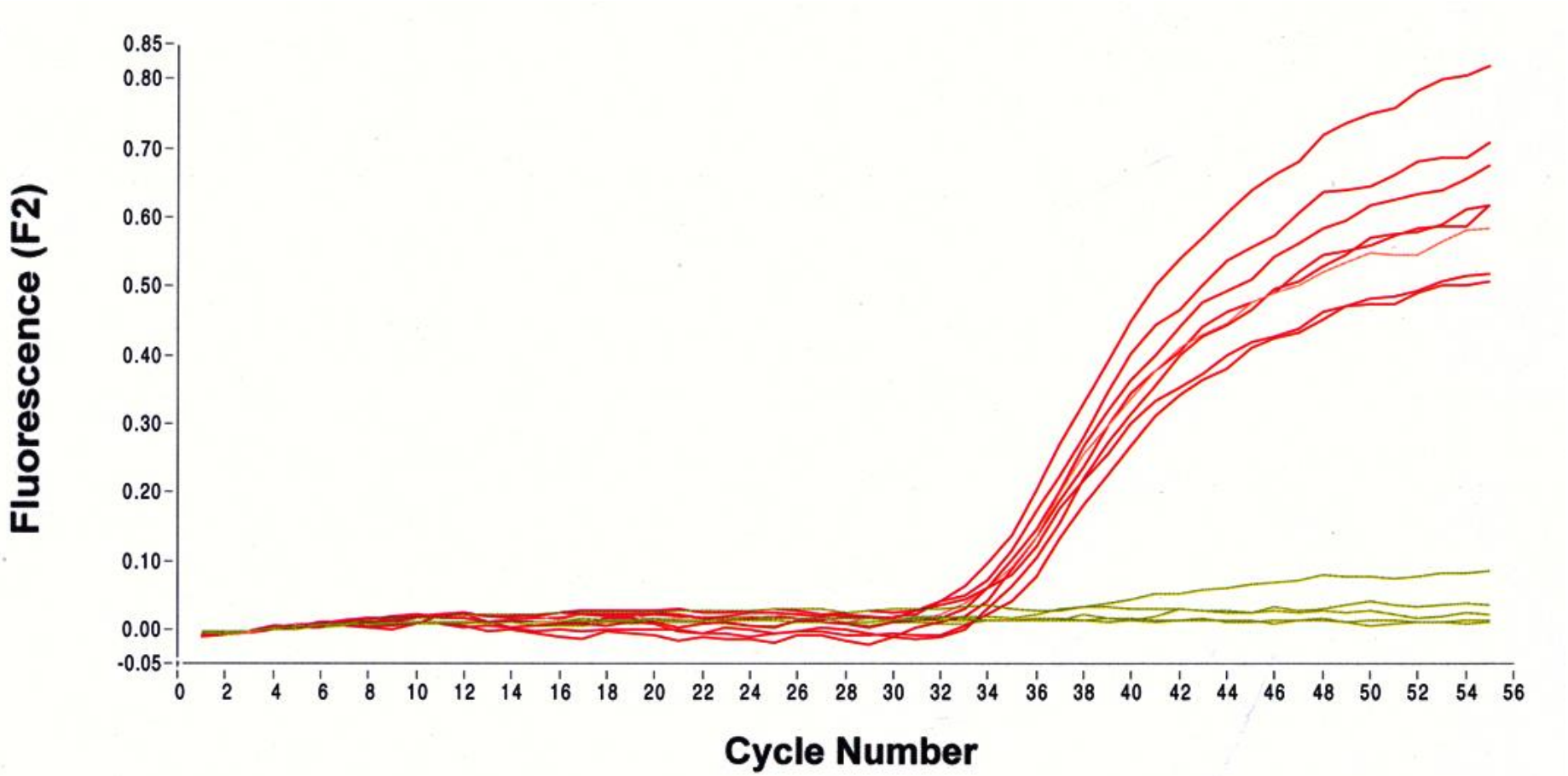
TaqMan Probe
- sequence specific
- fluorescent signal



Reference Gene Concentrations



TREC Concentrations

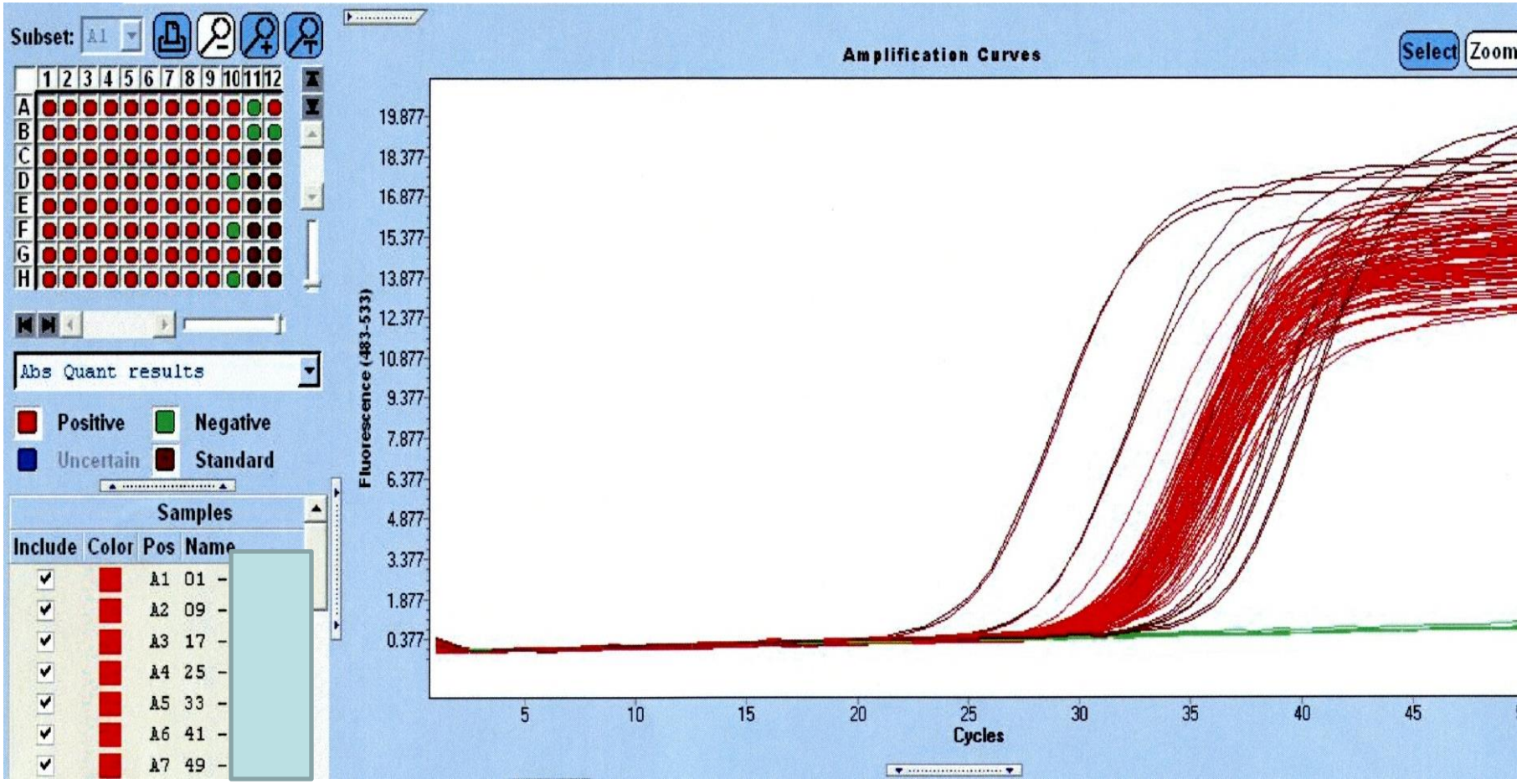


Utah Pilot Study

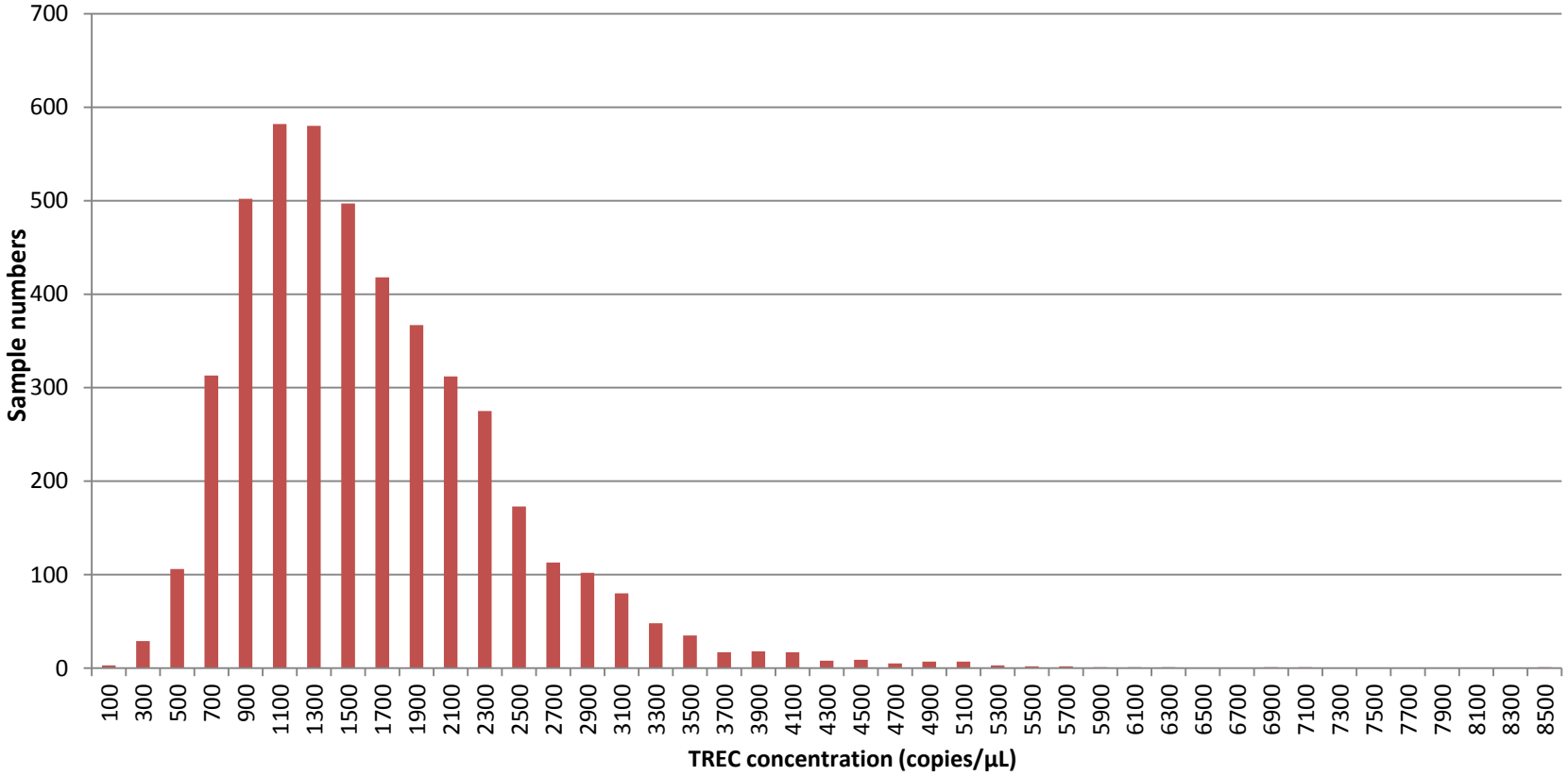
- 4,999 dried blood samples (DBS)
  - 4,665 non- NICU
  - 344 NICU

- TREC Singleplex Assay
  - TREC concentrations
  - β -actin concentrations (reference gene)

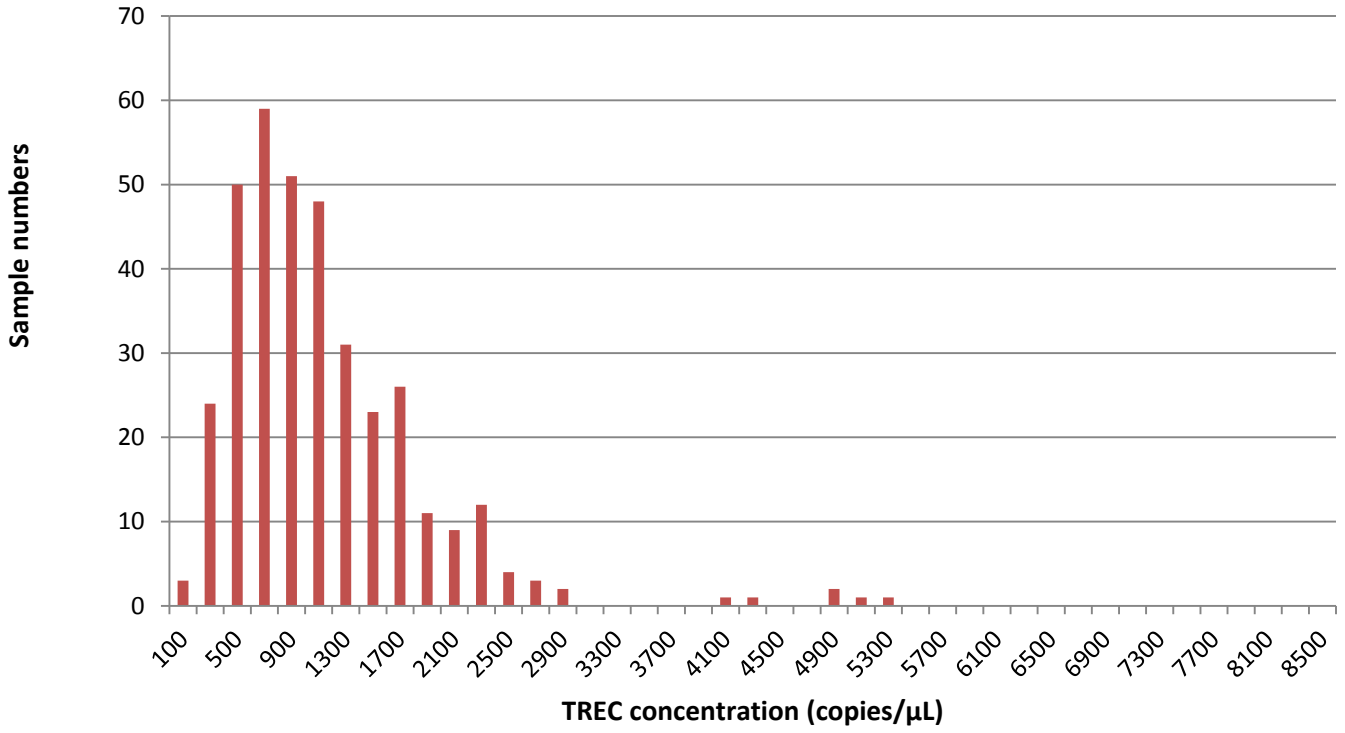
Results


Subset:
A1
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
Abs Quant results
Positive
Negative
Uncertain
Standard
Samples
Include
Color
Pos
Name
A1 01 -
A2 09 -
A3 17 -
A4 25 -
A5 33 -
A6 41 -
A7 49 -
Amplification Curves
Select
Zoom
Fluorescence (483-533)
19.877
18.377
16.877
15.377
13.877
12.377
10.877
9.377
7.877
6.377
4.877
3.377
1.877
0.377
5
10
15
20
25
30
35
40
45
Cycles

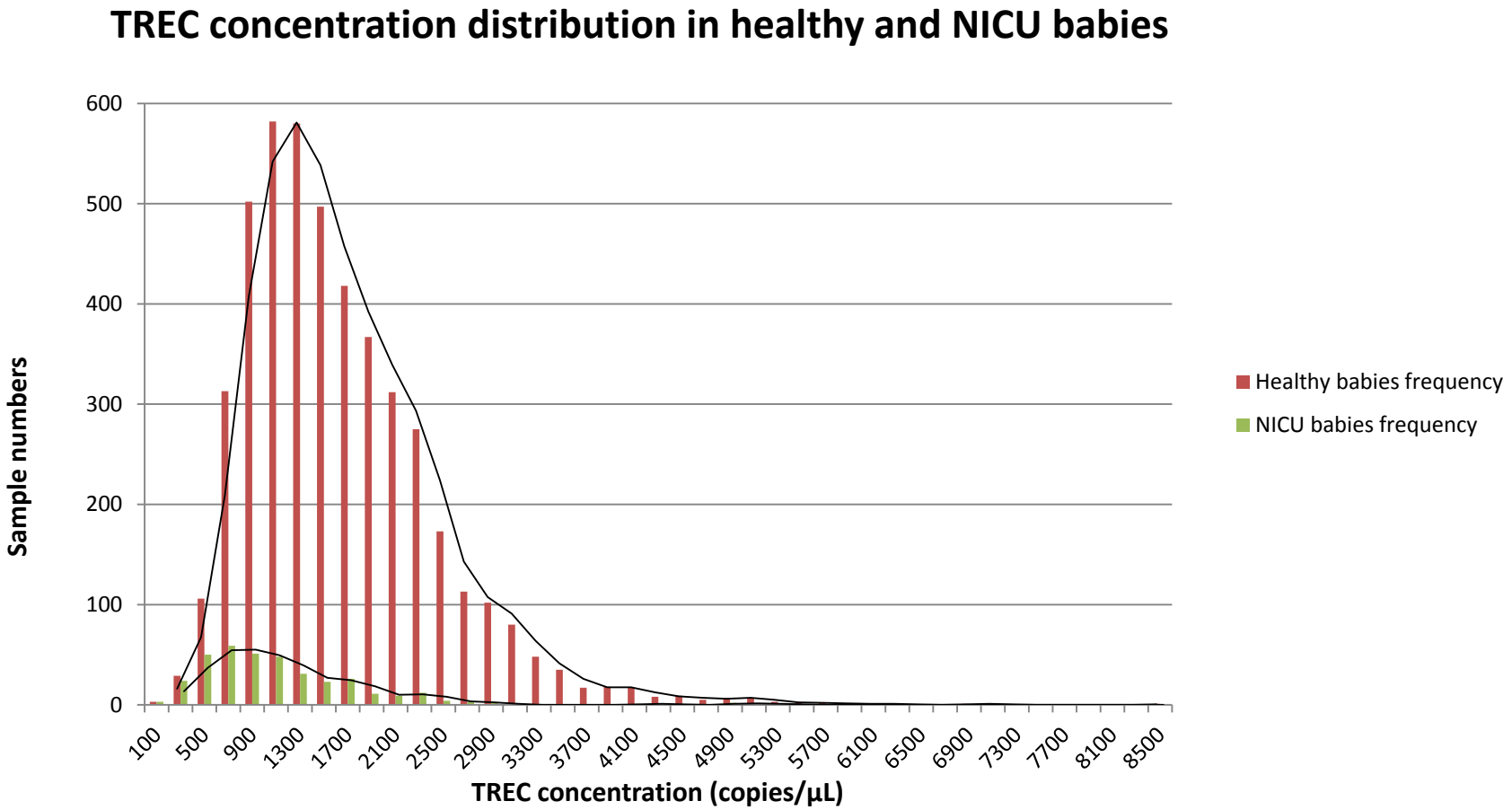
TREC Concentrations in Non-NICU DBS



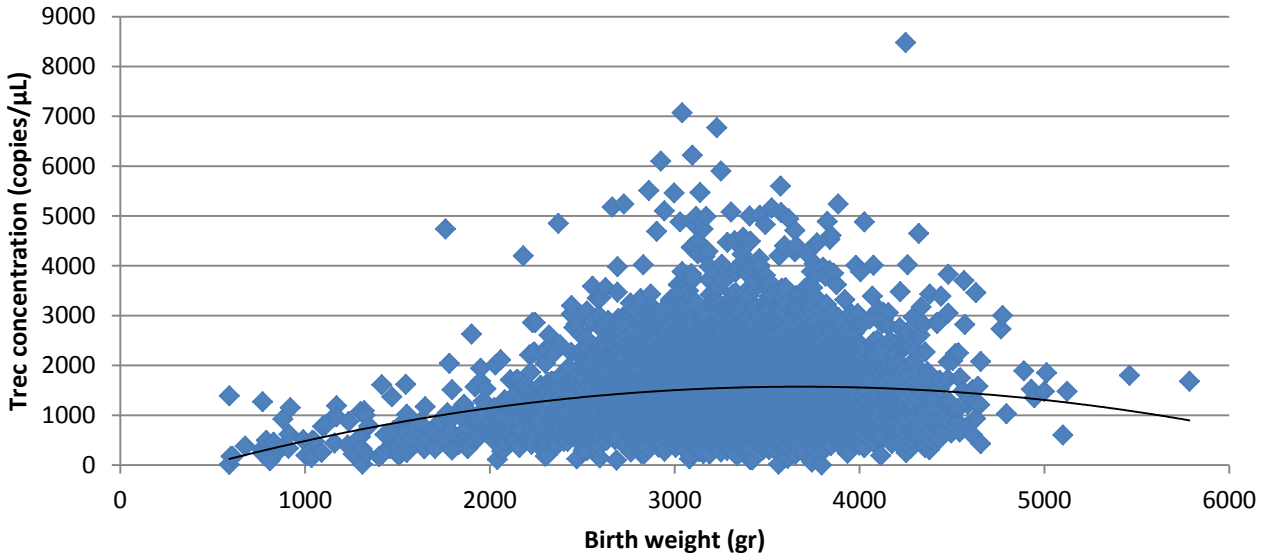
TREC Concentrations in NICU DBS

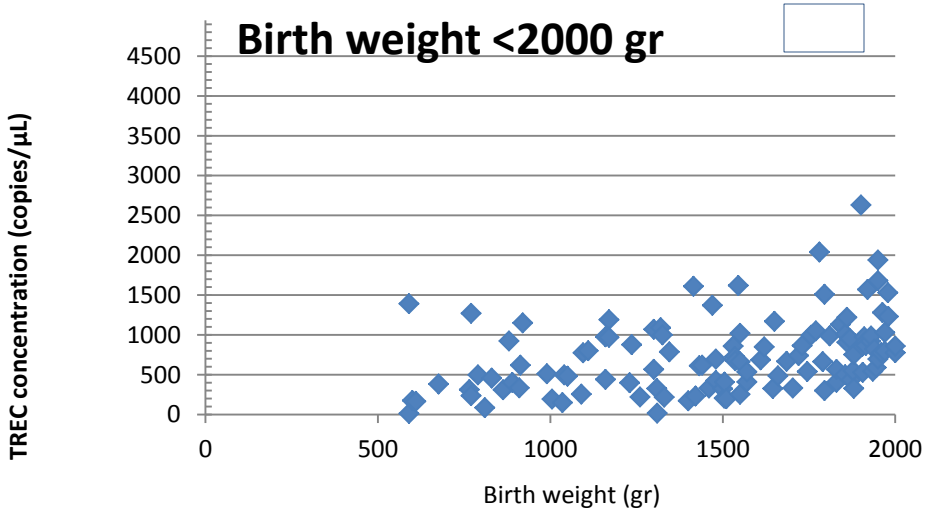


Pilot Study Results



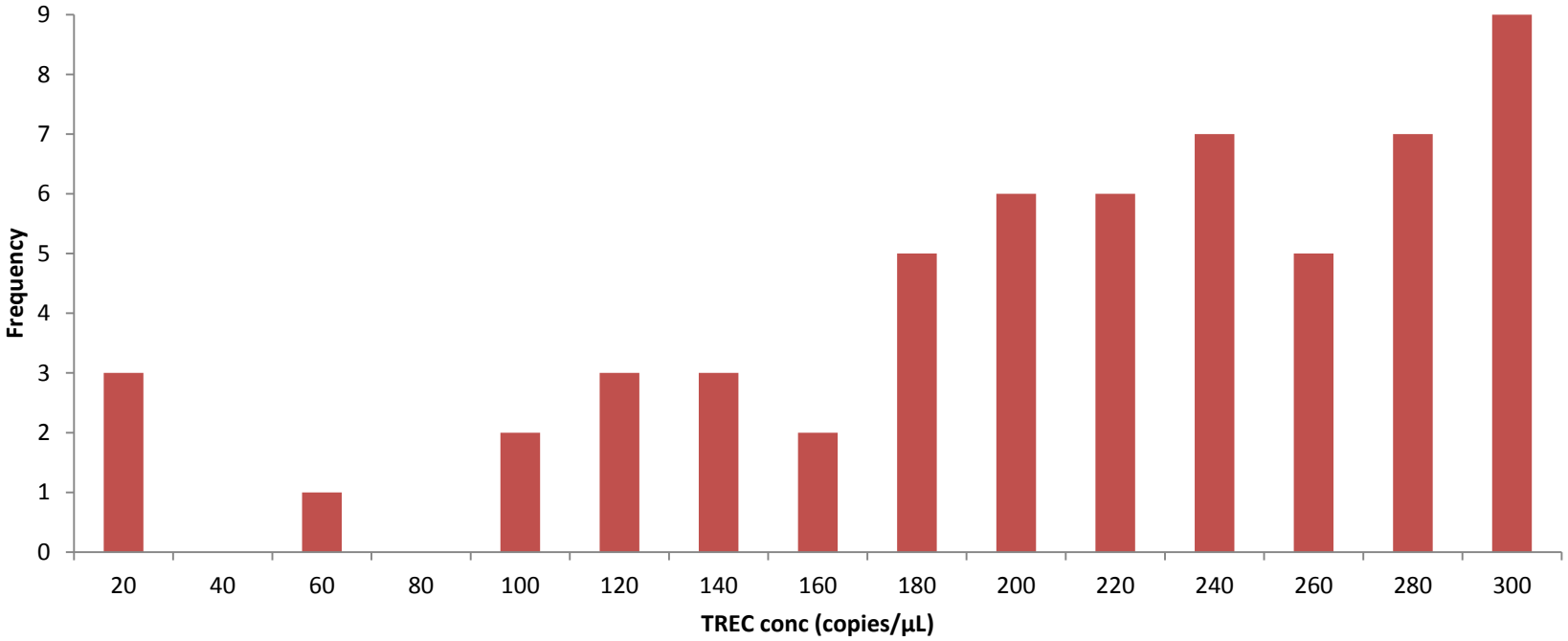
TREC Concentrations vs. Birth Weight





Low TREC Concentrations

TREC conc frequency count (59 samples with conc of up to 300 copies/uL, 1.2% of samples)



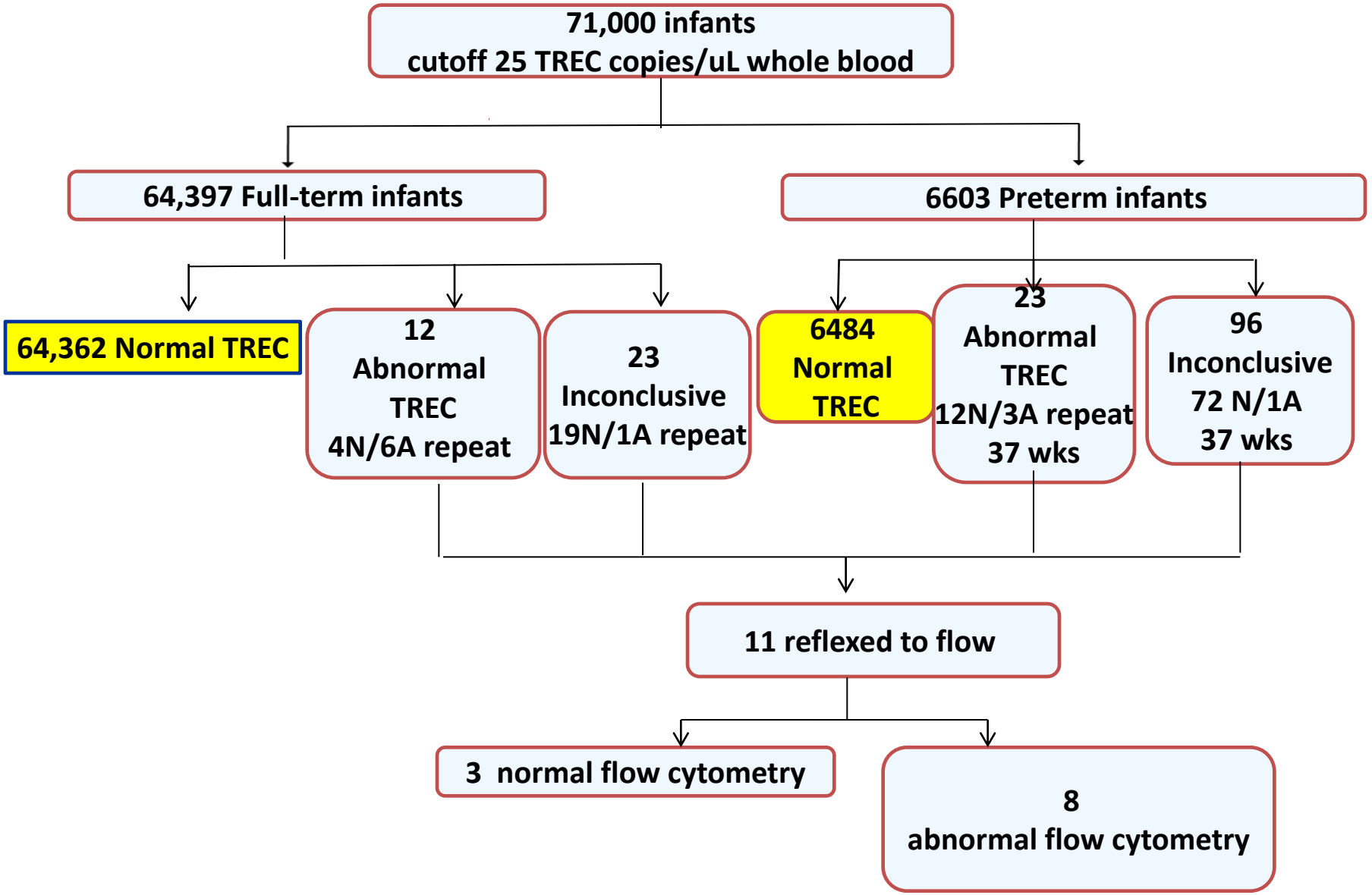
TREC Data Summary

NICU vs. Non-NICU	Number / %	TREC (copies/µL whole blood) mean	TREC (copies/uL whole blood) median	TREC (copies/µL whole blood) minimum	TREC (copies/µL whole blood) maximum
NICU	344 /6.9%	1035.3	865	11.8	5160
Non- NICU	4,655/93.1%	1538.4	1380	16.7	8480
Combined	4,999/100%	1503.8	1350	11.8	8480

Summary

- Cutoff - 100 TREC copies/uL whole blood
- Detects all CDC SCID positive DBS
- Detects SCID newborns
  - Reticular Dysgenesis, ADA
- 8 samples with low TREC required repeat testing
  - 6/8 resolved
  - 2/8 repeatedly below the cutoff – premature (590g,1310g)

Wisconsin



Adapted from Routes et al. JAMA 2010.

Massachusetts (Multiplex)Results

DNA Source	No. of patients	No. of assays performed on each sample	TREC copies/μL whole blood, mean	RNase P copies/μL whole blood, mean
Initial sample from all infants in cohort	25 609	1	1.9×10^3	7.1×10^4
Not NICU	23 667	1	2.0×10^3	7.2×10^4
NICU	1942	1	1.4×10^3	5.5×10^4
SCID control2 A	1	129	ND[a]	2.5×10^4
SCID control2 B	1	129	ND	2.4×10^4
SCID (IL7RA)[b]	1	3	ND	3.9×10^4
X-linked SCID	1	16	ND	1.6×10^4
SCID (unknown)	1	4	ND	3.3×10^4
SCID (ADA)[b]	1	4	ND	2.1×10^4
SCID (PNP)[b]	1	4	1.4×10[c]	2.9×10^4
SCID (unknown)	1	1	58[c]	4.8×10^4
X-linked SCID[d]	1	1	27[c]	3.2×10^4
X-linked SCID[d]	1	1	ND	4.8×10^4

Cutoff 252 copies TREC/uL

Thompson et al. Clin Chem.2010

Abnormal Results and Immunodeficiency

State	Start of Screening	Number of Months Screening	Annual Births or Number Studied	Number of Infants Screened as of April 30, 2011	SCID[a]	SCID Variant[b]	Non SCID[c]
WI	1/1/2008	40	69,232	243,707	4	0	7
MA	2/1/2009	27	77,022	161,707	1	0	14
Navajo Nation	2/1/2009	27	2,000	1,297	0	0	0
NY	9/30/2010	7	236,656	136,635	4	0	12
CA	8/1/2010	9	510,000	358,000	5	6	3
PR	8/1/2010	9	45,620	29,115	0*	0	3
LA	10/1/2010	7	65,268	31,464	0	0	1
	Total	126	1,005,798	961,925	14	6	40

*One infant with suspected SCID expired before diagnosis confirmed.

SCID - < 300 T-cells/uL

SCID Variant – "leaky SCID", Omenn Syndrome unknown gene defect (300-1,500 T-cell/uL)

Non SCID – immundeficiency not due to SCID, i.e trisomy 21

Adapted from executive summary report on SCID

Incidence (California)

Diagnostic Category	Race or Ethnicity	Incidence Rate	95% Confidence Intervals	
			Lower	Upper
SCID	All	1 in 33,000	1 in 20,000	1 in 65,000
SCID	Hispanic Only	1 in 22,000	1 in 9,000	1 in 40,000
All Related T-cell Lymphocyte Deficiencies	All	1 in 22,000	1 in 13, 300	1 in 35,000

Adapted from executive summary report on SCID

Clinical Characteristics of SCID Cases

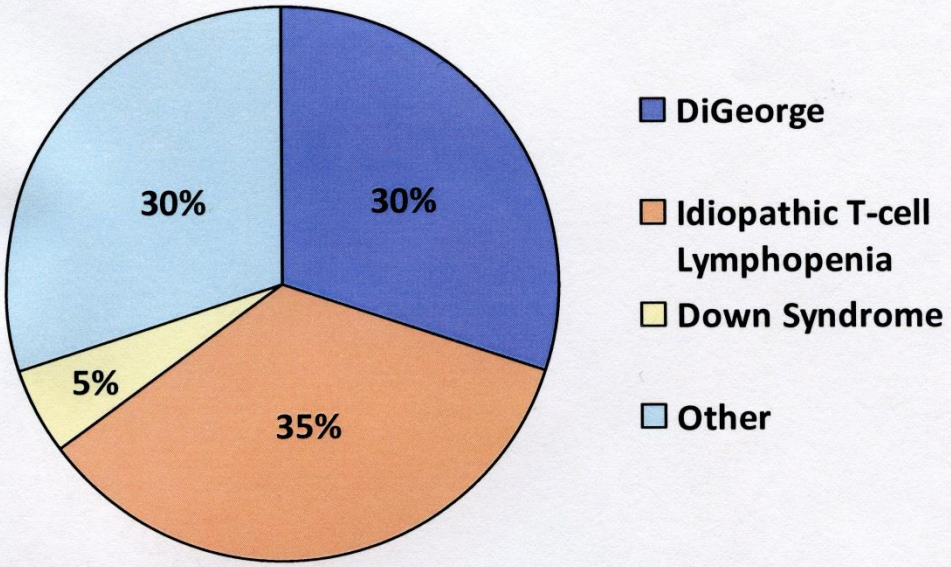
Characteristic		Number of SCID Cases (%)
Sex	Male	6 (67%)
	Female	3 (33%)
Molecular Type of SCID*	Autosomal Recessive (IL-7Ra)	2 (22%)
	Autosomal Recessive (RAG-1)	2 (22%)
	Autosomal Recessive (ADA)	2 (22%)
	X-Linked (IL2RG)	1 (11%)
Race or ethnicity	Hispanic	6 (67%)
	African American	2 (22%)
	Asian	1 (11%)

*Molecular typing on one case is pending.

Adapted from executive summary report on SCID

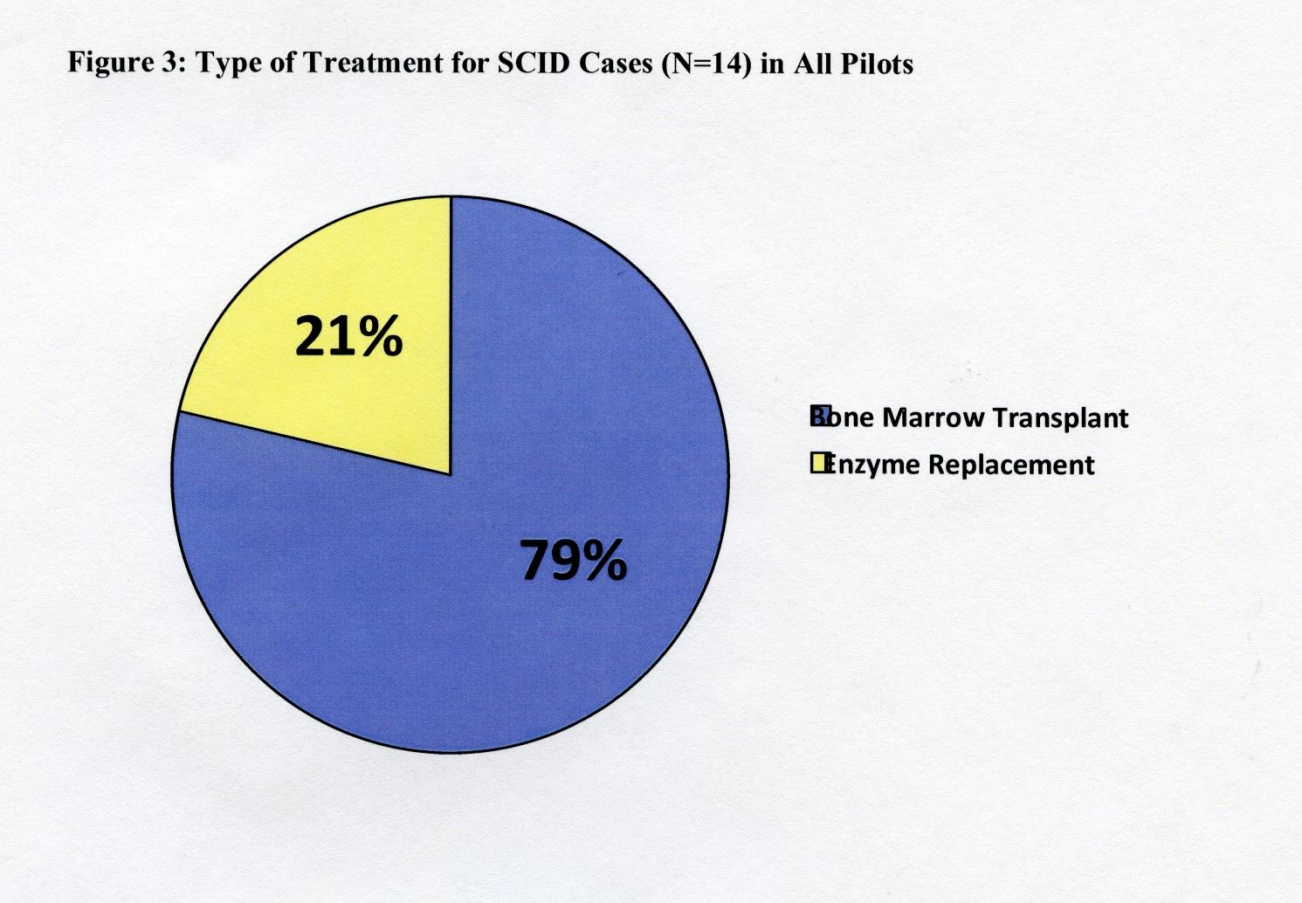
NON SCID Cases

Figure 4: Diagnosis for Non SCID Cases for All Pilots (N=40)



Adapted from executive summary report on SCID

Treatment



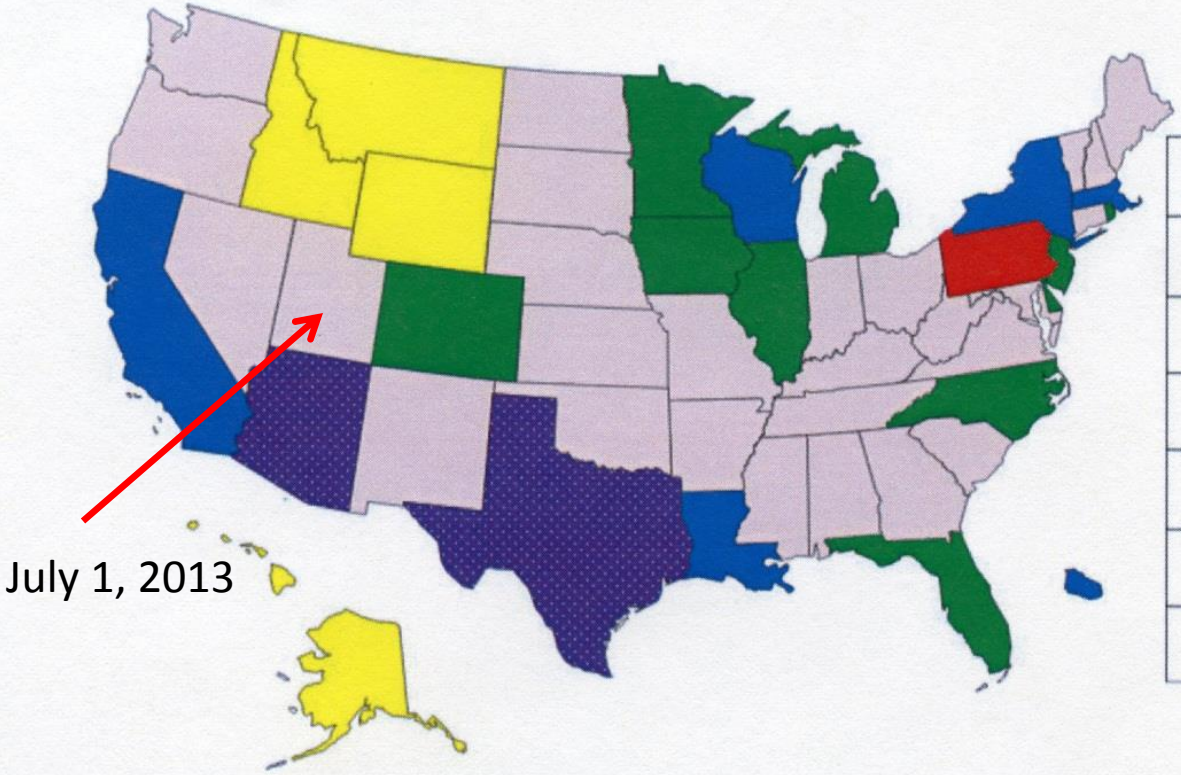
Adapted from executive summary report on SCID

Take Home Points

- SCID is fatal unless treated
- Prompt treatment for SCID infants significantly increases survival
- SCID infants do not have any symptoms at birth, making prompt diagnosis difficult
- T-cell receptor excision circles (TREC) assays are currently being used to screen newborns for SCID
- TRECs are not specific for SCID, but markers for T-cell lymphopenia, recent thymic emigrant T-cells
- Cut-off for TREC concentrations vary from state to state and are method dependent
- Screening for SCID with TREC – 100% sensitivity 98% specificity

National SCID Screening

Figure 5. Map of Newborn Screening for SCID Implementation Status



July 1, 2013

Number of States	Shading	Status
6		State-wide Screening
1		Partial Screening
2		Targeted Pilots
10		Screening Approved
28		Fact Finding
4		Regional Partner

Adapted from executive summary report on SCID

Acknowledgements

- **Utah Department of Health (UDOH)**

  Dr. Harper Randall

  Kim Hart

- **University of Utah/ARUP**

  Dr. Harry Hill

  Dr. Marzia Pasquali

  Dr. Noriko Kusukawa

  Dr.Carl Wittwer

  Dr. Orly Ardon

  Jorja Warren

  Wei Xie

  Mike Graczyk

  Andy Lorance

- **Wisconsin State Laboratory of Hygiene**

  Dr. Mei Baker

- **Primary Children”s Medical Center**

  Dr. Karin Chen

Thank you!


Mosby
ISSN 0091-6749
VOLUME 120 NO. 4
OCTOBER 2007
www.jacionline.org
THE JOURNAL OF
Allergy AND Clinical
Immunology
We Beat
SCID!

Quantification (Standard Curve)